UNITED NATIONS

SECRETARIAT

ST

Distr.
GENERAL

ST/SOA/SER.J/22
30 April 1976

Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER BRACTEATUM

No. 22

PRECURSOR INCORPORATION EXPERIMENTS IN PAPAVER ALKALOID BIOSYNTHESIS

I. PAPAVER BRACTEATUM LINDL.

by: Arnold Nordal, Berit Smestad Paulsen and Jens K. Wold,

Institute of Pharmacy,
Department of Pharmacognosy,
University of Oslo,
Norway.

GE. 76-5110

PRECURSOR INCORPORATION EXPERIMENTS IN PAPAVER ALKALOID BIOSYNTHESIS

I. PAPAVER BRACTEATUM LINDL. [1]

It has been known for several years that different species or strains of the fungus Penicillium are able to incorporate precursors and that the yield of penicillin is increased when suitable precursors are added to the growth medium (1). It has also been shown that weed-killers of the hormone type are able to penetrate into the aerial parts of plants when solutions of such compounds are sprayed on the fields (2). These observations led us to investigate whether the same principle could be used for the introduction into alkaloid-producing plants of nitrogenous compounds, such as amino acids dissolved in a surface active agent. If so, we assumed that the compounds would enter the metabolic pathway of the plants and act as precursors in the biosynthesis of alkaloids.

Phenylalanine, tyrosine, glycine and urea, all uniformly labelled with ^{14}C, were chosen as the precursors to be studied. The experiments were carried out using Papaver bracteatum Lindl. (Arya II) as test organism. After exposure of the precursors to the plant surface for a certain time, the plants were extracted with ethanolic ammonia and the low molecular weight radioactive compounds separated by TLC and located by radioautography. The radioactivity of the unconverted isotopes (A), of the radioactive metabolites (B), and of the radioactive thebaine (C) were measured by liquid scintillation counting and the results presented in a diagram (Table 1).

1/ The present study forms part of the international research programme organized by the United Nations Narcotics Laboratory at the request of the Commission on Narcotic Drugs. The objective of this programme is to increase the yield of phenanthrene alkaloids per unit of cultivated area in order to facilitate control and to ensure adequate supplies of codeine for medical purposes.

EXPERIMENTAL PROCEDURE

Plant material. For the experiments, we used Papaver bracteatum Lindl. (Arya II), grown in the Botanical Garden, University of Oslo, Tøyen, from seeds kindly provided by Dr. O.J. Braenden, U.N. Narcotics Laboratory, Geneva. At the start of the experiments, the plants had shed their petals, but the capsules were still fresh and green (Figures 1 and 2).

Labelled precursors. Uniformly ^{14}C labelled compounds were used (250 μ Ci of each): L-phenylalanine, L-tyrosine, L-glycine as hydrochlorides and urea, all supplied by New England Nuclear. Before use, the solutions of the amino acids were neutralized with sodium hydrogen carbonate, freeze-dried and dissolved in 1.5 ml of a 1% solution of the surfactant: MON 0011, Monsanto Company, St. Louis, Miss., U.S.A., kindly supplied by Dr. Kåre Lund-Høye, The Agricultural University of Norway, Ås. The urea was freed from ethanol by evaporation and then dissolved in 1 ml of the same surfactant solution as above.

Application of labelled precursors. Each of the precursors was applied to separate plants. The solutions of phenylalanine, tyrosine, and urea were applied onto the upper part of the stem and to the four upper leaves. Glycine was applied onto the capsule. After application of the isotopes, the plants were covered with plastic bags having the corners cut off. This was done to prevent rain from washing the isotopes off the plants and to prevent condensation of water inside the bags (Figure 2). The plants were left photosynthesizing from August 8 to September 3, 1975. During this period the weather was warm and sunny in the Oslo area, for which reason the plants were irrigated carefully a few times. After harvesting, the plants were scanned for radioactivity using a "Nardeux" Geiger-Muller-counter. Radioactive material appeared to be present in all parts of the plants except the root. The following parts of the plant were analysed for their content of radioactive material: capsule, stem, leaves and root-stem transition.

Extraction of the plants. The whole capsule, parts of stem, leaves and root-stem transition from the four treated plants were extracted separately with ethanolic ammonia (5%) at about 70°C. After filtration and concentration, the extracts were subjected to TLC using systems 1 and 2.

TLC plates. Polygram Sil G/UV 254 from Macherey-Nagel & Co. (20 x 20 cm). The compounds were visualized by means of UV-light.

Thin layer chromatography (TLC) was carried out in the following solvent systems: 1. toluene: acetone: ethanol: 25% ammonia (45:45:7:3) vol/vol; 2. ethyl acetate: methanol: 25% ammonia (85:10:5) vol/vol, freshly prepared.

Radioautography. The TLC plates were exposed to Kodak RP X-omat Medical X-ray film RP54 for about two weeks before development. The radioactive compounds were thus located on the TLC plates (Figures 3 and 4) and the appropriate areas cut out and subjected to liquid scintillation counting.

Liquid scintillation counting was carried out in a Mark II liquid scintillation system, Nuclear - Chicago. As scintillant the following solution was used: PPO 5 g., Dimethyl POPOP 0.3 g., Toluene 1 l.

RESULTS AND DISCUSSION

The experiments show that phenylalanine, tyrosine, glycine and urea are able to penetrate Papaver bracteatum when dissolved in a 1% aqueous solution of a certain surfactant. From Table 1 it can be seen that a relatively high percentage of the labelled precursors has been converted into radioactive metabolites extractable with ethanolic ammonia.

As expected, radioactive thebaine was found in the plants treated with phenylalanine and tyrosine. However, it was also found that glycine and urea gave rise to radioactive thebaine (Figure 3, a-d, and Table 1). The figures show that several radioactive metabolites were formed, and also that very little radioactivity was present in the root-stem transitions and in the leaves of the four plants. No radioactive compounds were found in the roots. The non-metabolized amino acids and urea will remain at the place of application on the TLC-plates as they do not move in the solvent systems employed.

In order to avoid injury to the plants before they had reached a certain degree of maturity, the labelled precursors were applied at a late stage of development of the poppy plants. Another reason for the time chosen was that

the alkaloid biosynthesis was assumed to be most active during the period of development when the latex production of the plant was at its highest.

TABLE 1

(See Figure 3, a-d, and Figure 4)

	PHENYLALANINE		TYROSINE		GLYCINE		UREA	
	$\frac{Bx100}{A+B}$	$\frac{Cx100}{A+B}$	$\frac{Bx100}{A+B}$	$\frac{Cx100}{A+B}$	$\frac{Bx100}{A+B}$	$\frac{Cx100}{A+B}$	$\frac{Bx100}{A+B}$	$\frac{Cx100}{A+B}$
Capsule	57.4	13.6	25.8	1.2	50.2	3.7	56.3	2.4
Leaves	43.8	3.8	41.4	1.1	12.6	0.3	72.8	3.0
Stem	8.0	1.2	28.6	0.9	13.7	0.8	34.5	0.8
Root-stem transition	54.0	3.4	36.8	5.6	24.6	0.9	32.5	1.6

A: C/pm of unconverted precursor remaining at the starting line of the chromatogram.

B: C/pm of radioactive metabolites derived from the precursor.

C: C/pm of thebaine.

The figures under the headings $\frac{Bx100}{A+B}$ show the percentage of radioactive metabolites, and $\frac{Cx100}{A+B}$ the corresponding percentage of radioactive thebaine found in the extracts.

Although preliminary, the present experiments show clearly that amino acids and, even more important, urea applied to leaf and stem surfaces of P. bracteatum are absorbed by the plant and utilized in part in alkaloid biosynthesis.

REFERENCES

1. Pratt, R. and J. Dufrenoy: Antibiotics. J.B. Lippincott Company, Philadelphia, 1953.

2. Ashton, Floid M., and Alden S. Crafts: Mode and Action of Herbicides. Wiley Interscience Publications, 1973.

Figure 1 - Papaver bracteatum

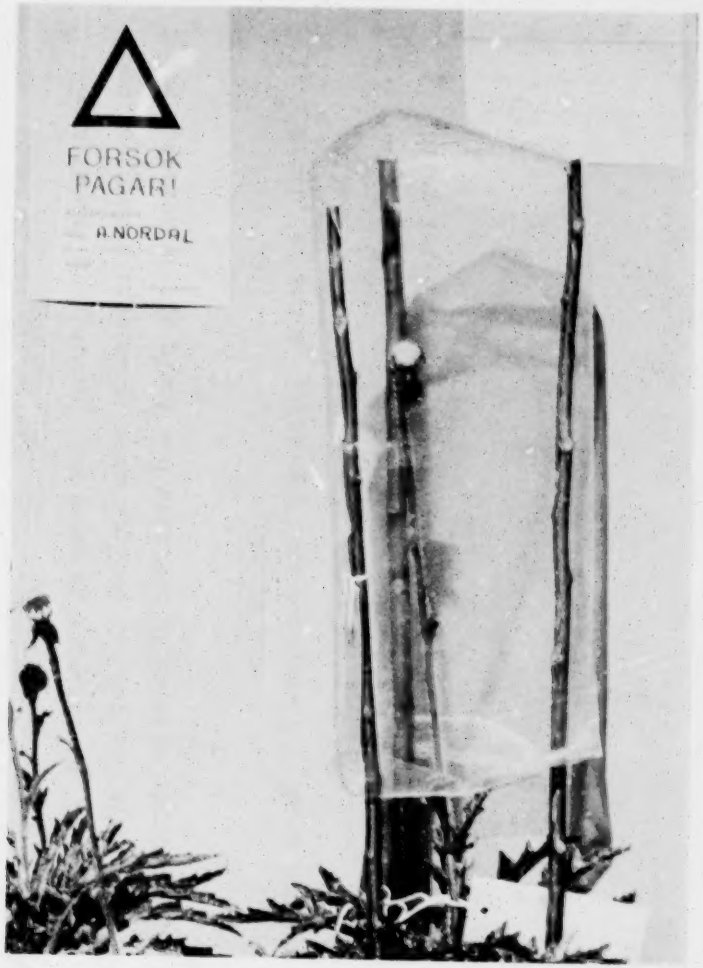

Figure 2 - Running experiment

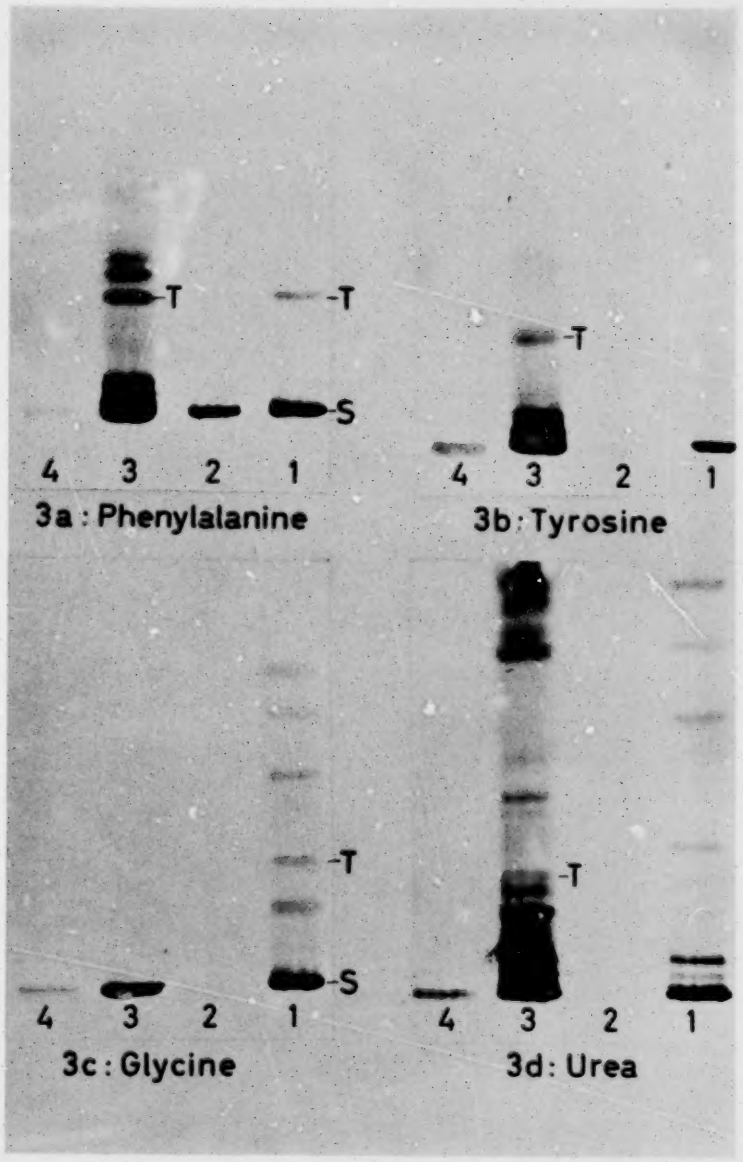

Figure 3 - Incorporation of different precursors into Papaver bracteatum. The numbers 1, 2, 3 and 4 show radioautograms of TLC plates derived from extracts of capsules, leaves and stems, and from the root-stem transition of the plants. "S" signifies the starting line and "T" the location of thebaine on the chromatograms.

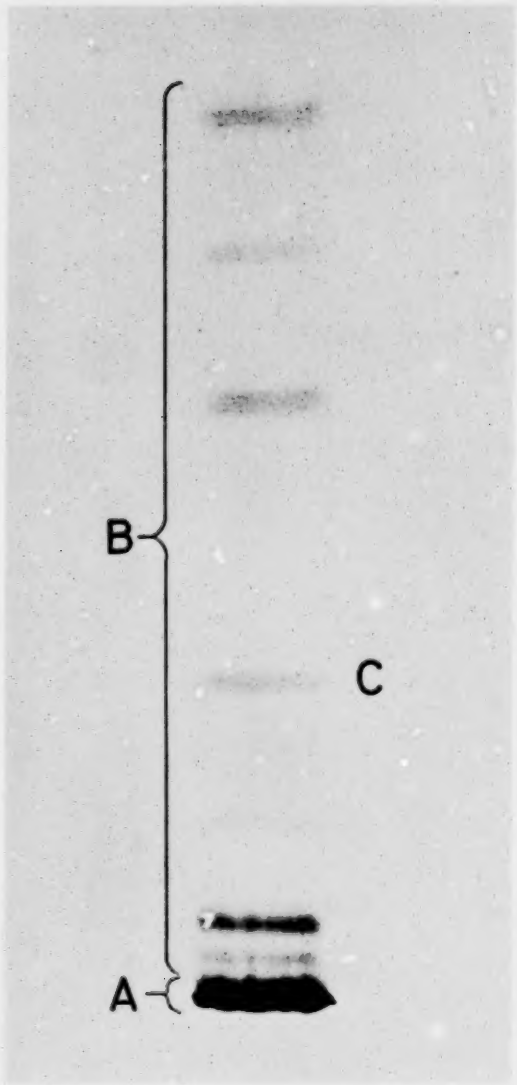

Figure 4 - Radioautogram of extract from Papaver bracteatum
(See Figure 3 C and Table 1).

A. Unconverted isotope remaining at the starting line.

B. Radioactive metabolites derived from the precursor.

C. Location of thebaine.